

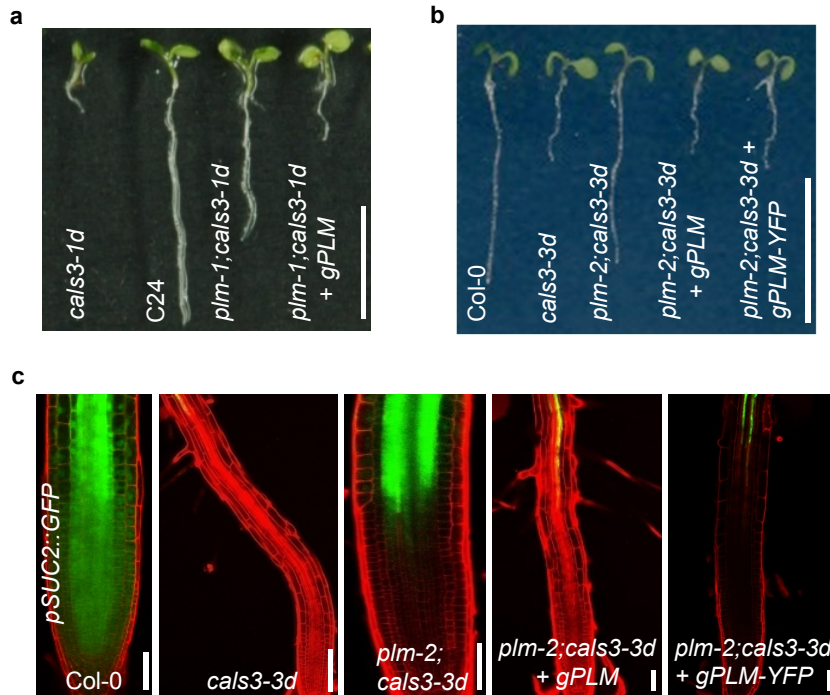
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Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading

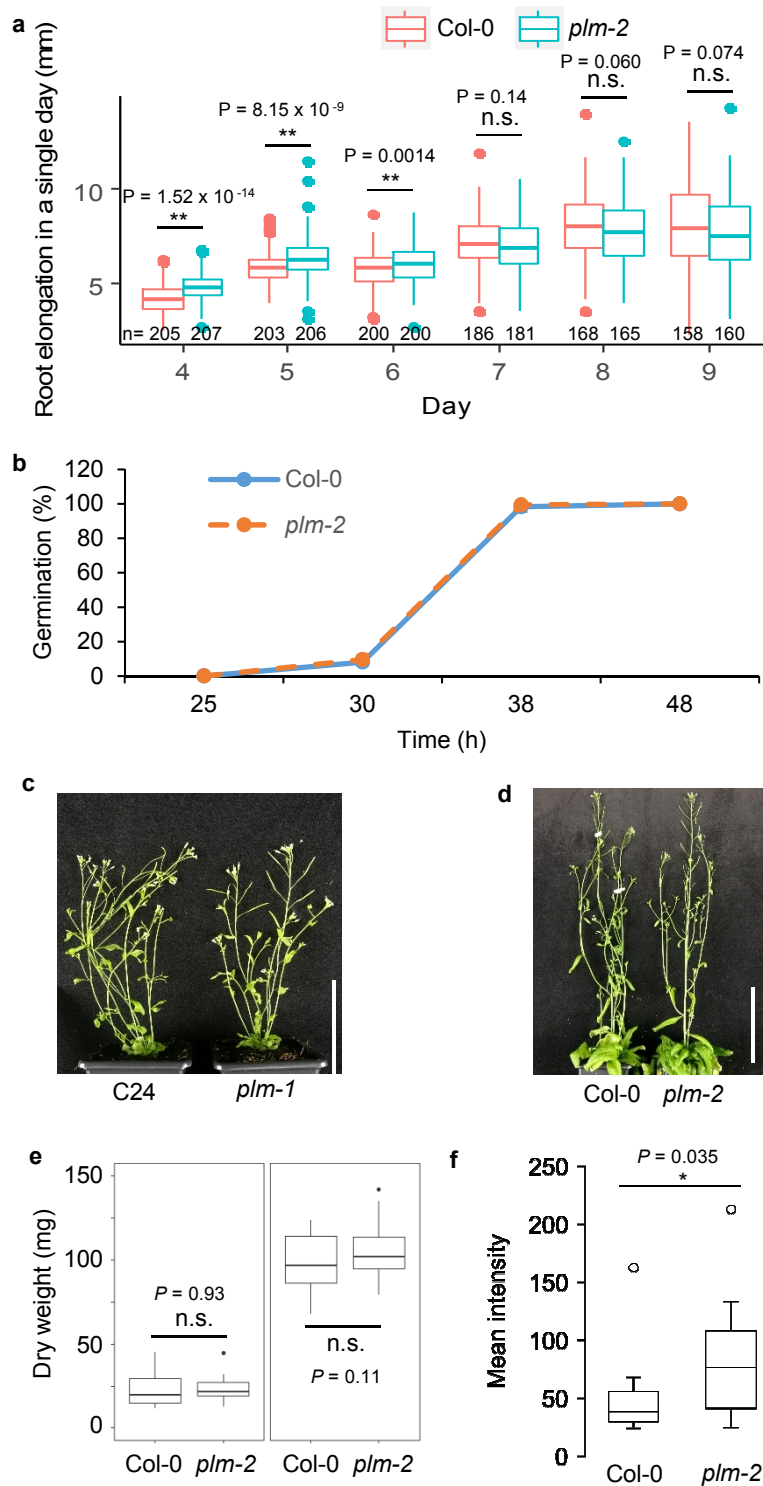
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Supplementary Fig. 1. *plm-2* rescues *calS3-3d* in Col-0 background, as well as *plm-1* in C24 background. a. Seedling phenotype of wild-type C24, *calS3-1d*, *plm-1;calS3-1d* and *plm-1;calS3-1d* transformed with PLM genomic insert. The experiment was repeated independently three times with similar results. b. Seedling phenotype of wild-type Col-0, *calS3-3d*, *plm-2;calS3-3d* and *plm-2;calS3-3d* transformed with PLM genomic and YFP-fusion inserts. c. *pSUC2::GFP* in the roots of wild-type Col-0, *calS3-3d*, *plm-2;calS3-3d* and *plm-2;calS3-3d* transformed with PLM genomic and YFP-fusion inserts. All the experiments were repeated independently three times with similar results. Scale bars, 1 cm (a, b), 50 μm (c).



Supplementary Fig. 2. Root elongation and plant biomass assays. a.

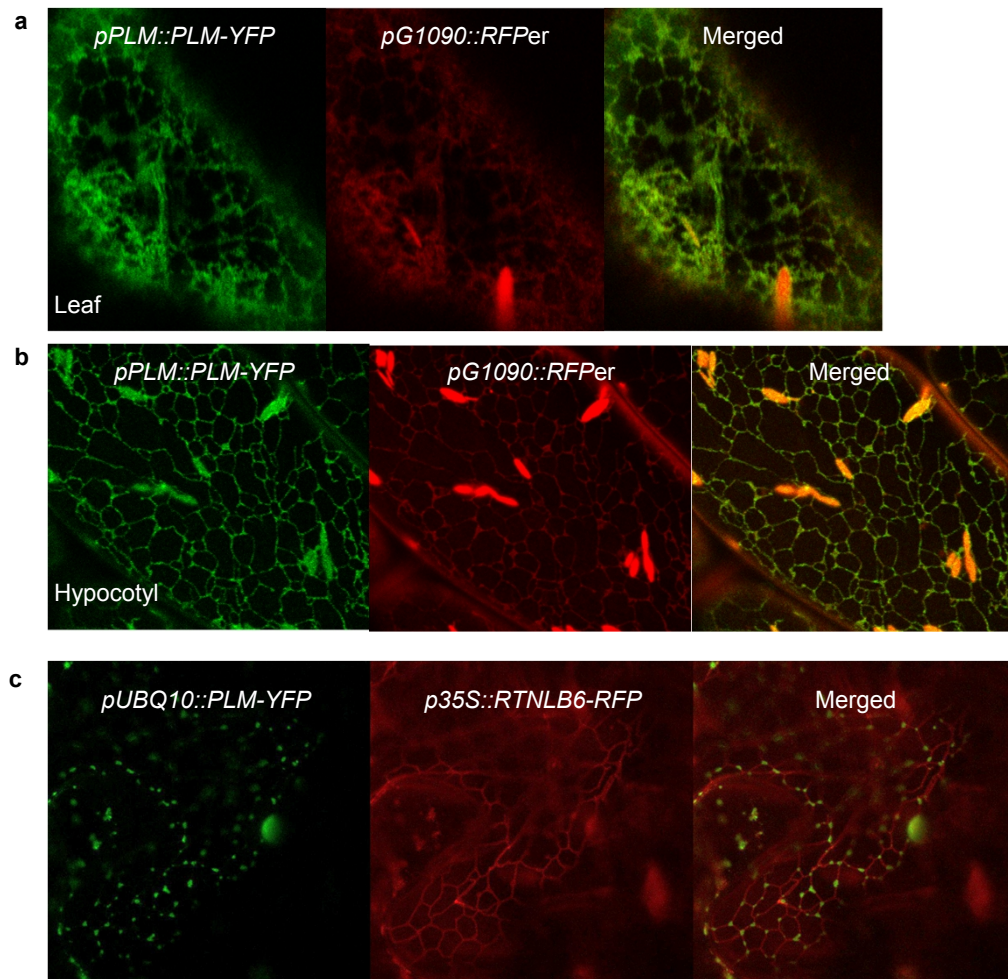
Root elongation in every single day of Col-0 and *plm-2*. The numbers of biologically independent plants are indicated in the graph. **b.** Germination rate of Col-0 and *plm-2* seeds. Data are based on 3 independent biological replicates with total 160 seeds. **c.** Adult plants of C24 and *plm-1*. This experiment was repeated independently three times with similar results. The linear model was used for test. **d.** Adult plants of Col-0 and *plm-2*. This experiment was repeated independently three times with similar results. **e.** Root (left panel) and shoot (right panel) biomass of adult plants at terminal flowering stage. $n = 29$ (both Col-0 and *plm-2*) biologically independent plants. **f.** GFP intensity in the root tip of 3-day-old seedlings. $n = 14$ (Col-0), $n = 13$ (*plm-2*) biologically independent plants. In the box plots, the boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values. The black lines within the boxes indicate the median values. Outliers are shown as dots. Significant differences were determined by two-tailed Mann-Whitney-Wilcoxon test. ** $P < 0.01$. n.s., no significance.

PLM	-----	0
SMS1	MLSA STMKEVYVWSPKKVADWLL ENAMPEYCEPLE---HFTGQDLINLTQEDFKKPPLCR	57
SMS2	-----	0
SMSr	-MAGPNQLCIRRWTTKHVAVWLKDEGFFEYVDILCNKHRLDGITLLTLEYDLRSPPLEI	59
PLM	-----MTK-----	3
SMS1	VSSDNGQRLLDMIETLKM EHHLEAHKNGHANGHLNIGVDIPTDG---SFSIKIKPNGM	113
SMS2	-----MDIETAKLEEHLNQSPDPTNTYARPAE--PVEE-----NKNNGKPKSL	45
SMSr	KVLGDIKRLMLSVRKLQKI-HIDVLEEMGYNSDSPMGSMTPFISALQSTDWLCNGELSHD	118
PLM	-GGLGIAAMSYVVIDYMRYSVPVWHSRLMPVLWSVLAIAVVTRVLFYKHSKELRAAIPF	62
SMS1	PNGYR-----KEMIKIPPELERSQYPMEW-----GKTFLAFLY-	147
SMS2	SSGLR--KGTKKYPDYIQIAMPTESRNKFPLEW-----WKTGIAFIY-	85
SMSr	CDGPITDLNSDQYQ-YM-NGKNKHSVRRLDPEY-----WKTILSCIY-	158
	. * : : * : :	
	<u>D1</u>	
PLM	LGSIVFLLCALLFEALCVRSVTAVLGLDWHRETPLPDTGQWFLALNESLPGLVEILR	122
SMS1	-----ALSC-FVLTVMISVV--HERVPPKEVQPLPDTFFDHFNRVQW--AFSICEING	197
SMS2	-----AVFN-LVLTVMITVV--HERVPPKELSPPLPKFFDYIDRVKW--AFSVSEING	135
SMSr	-----VFIV-FGFTSFIMVIV--HERVPMQTYPLPDIFLDSVPRIPW--AFAMTEVCG	208
	. : : : : * : . ***** : : *	
	<u>D2</u>	
PLM	AHIIGLHHFLMLFIMLGFSVVFDSVKAPGLGLGARYIFTMGVGRLLRAITFVSTILPSAR	182
SMS1	MILVGL--WLIQWLLKY-----KSIISRRFFCIVGTLYLRCITMYVTTLPVPG	245
SMS2	IILVGL--WITQWFLRY-----KSIIVGRRFFCIIGTLYLRCITMYVTTLPVPG	183
SMSr	MILCYI--WLLVLLHKKH-----RSILLRRLCSLMGTVFLLRCFTMFVTSLSVPG	256
	: : : : : . : * : * . * : * *	
PLM	--PWCASARFNNVPSQPHRWAQKYVPYANDPAAIRKLLHWDAAAYADPGSYIGDYRADWG	240
SMS1	MHFNCSPKLFGDWEAQLRRI-----MKLI-----	269
SMS2	MHFQCAPKLNQDSQAKVQRI-----LRLI-----	207
SMSr	QHLQCTGKIYGSVWEKLHRA-----FAIW-----	280
	* : . . : *	
	<u>D3</u>	
PLM	SMSFLEFLRPSYSEGSSWFALLKKAGGGCNDLMYSGHMLVAVLTAMAWTEA---YGGFS	297
SMS1	-----AGGGLSITGSHNMGDYL YSGHTVMLTLTYLFIKEYSPRRLLWY	313
SMS2	-----SGGGLSITGSHILCGDFLFSGHTVTLTLTYLFIKEYSPRHFWWY	251
SMSr	-----SGFGMTLTGVH--TCGDYMFSGHTVVLTMLNFFVTEYTPRSWNFL	323
	: : : . * : : * : : : . *	
	<u>D4</u>	
PLM	SAMIWLFVAHSAQREIRERHHYTVDCIVAIYVGILLWKMTGFIWSAERKTKQTKLEKIQN	357
SMS1	HWICWLLSVVGIFCILLAH DHYTVDVVVAYYITTRLFW-----WYHTMANQQVLKEASQM	368
SMS2	HLICWLLSAAGIICILVAEHYTDVIAIYYITTRLFW-----WYHSMANEKNLKVSSQT	306
SMSr	HTLSWVLNLFGIFFILAAEHYSIDVFI AFYITTRLFL-----YYHTLANRAYQQSRR-	377
	: * : . : : * : * : * : : : :	
PLM	SLI-----HAAKDGDIE TVRRL--V-EEIEVSSRVEKQSKVISNRTMTVFACA	402
SMS1	NLLARVWYRPFQYFEKNVQGI VPRSYHWPFPWPVHLSRQV--KYSRLVNDT-----	419
SMS2	NFLSRWWFPIFYFFEKNVQGSIPCCFSWPLSWPPGCFKSSCKKYSRVQKIGEDNEKST-	365
SMSr	----ARIWFPMFSEFCNVNGTVPNEYCWPFKPAIMKRLIG-----	415
	. : *	
PLM	TVITTLTIVILALTLSDG	421
SMS1	-----	419
SMS2	-----	365
SMSr	-----	415

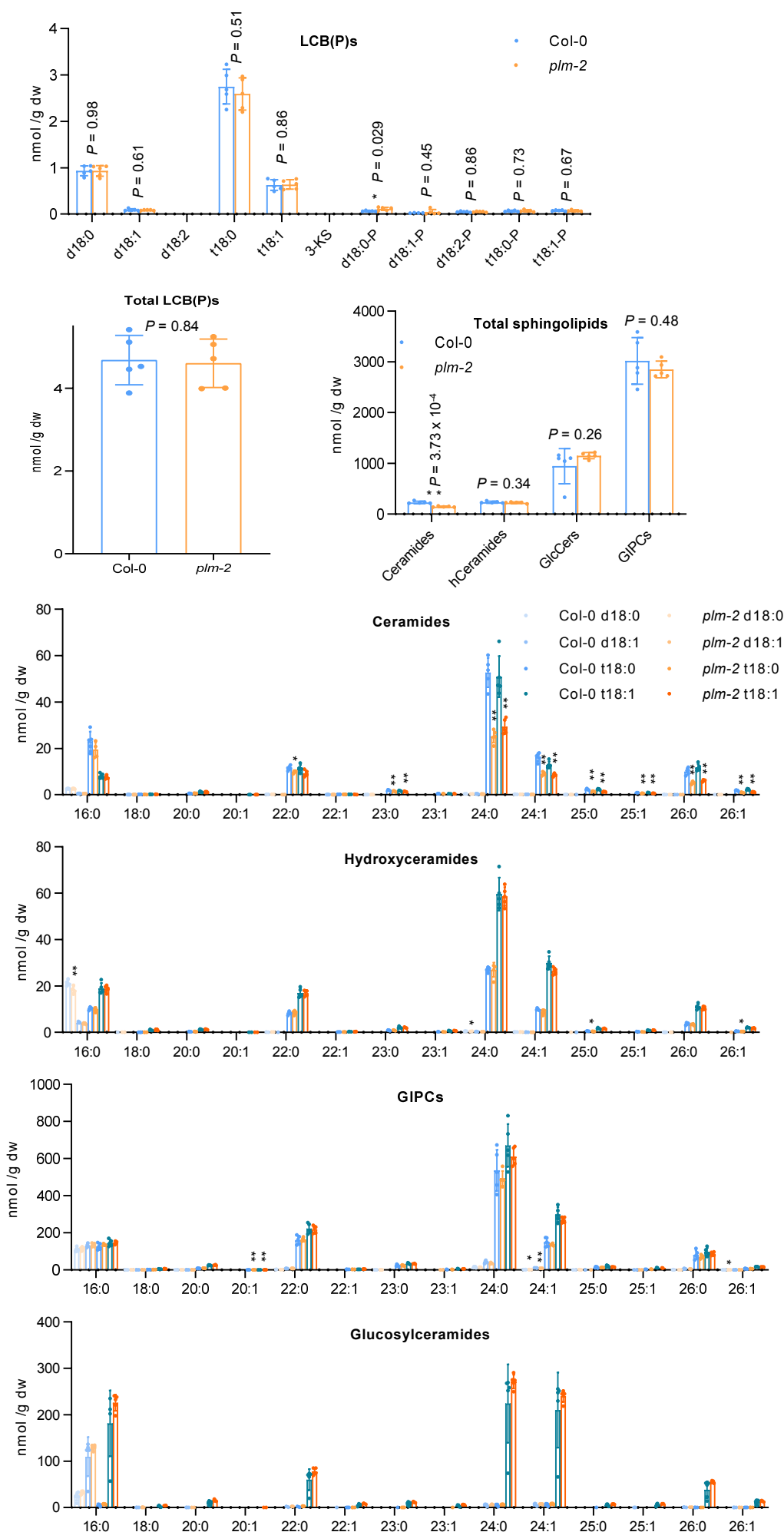
Supplementary Fig. 3. Protein Sequence alignment of PLM (At1g43580) and HsSMSs. Four conserved domains (D1, D2, D3 and D4) within human SMSs are highlighted with black lines. H-H-D residues that form a catalytic triad are highlighted by blue arrows. *, identical residues. ., similar residues.

PLM	MTKGGGLGIAAMSYVVIDYMRYVSPVWHSRLMPVLWSVLAIAVVTRV-LFYKHWSKELRAA	59
IPCS1	-----MTLYIRREASKLWRRFCSEITTEIGLLAENWK-----	32
IPCS2	-----MTLYIRRESSKLWKRFCSEISTEIGLLAENWK-----	32
IPCS3	-----MPVYVDREAPKLWRRRIYSEATLEASLLAEKWK-----	32
	:: * ** : . * : ::*.	
PLM	IPFLGSIVFLLCALLFEALCVRSVTAVLGL-DWHRET PPLPDTGQWFLALNESPGLTV	118
IPCS1	-----YLLAGLLCQYI--HGLA-ARGVHYIHRPGPTLQDSGFFVLPELGQDKG-FIS	80
IPCS2	-----YLLAGLICQYI--HGLA-AKGVHYIHRPGPTLQDLGFFLLPELGQERS-YIS	80
IPCS3	-----LVLAGLVFQYI--HGLA-AHGVHYLHRPGPTLQDAGFFILPALGQDKA-FFS	80
	:*..* : : : : : : . * : ** * * * * : * * : . : :	
PLM	EILRAHIIGLHHFLMLFIMLGFS-VVFD SVKAPGLGLGARYIFTMGVGRLLRAITFVSTI	177
IPCS1	ETVFTCV-----FL-SFFLWTFHPFIVKSKKIYTVLIWCRVLAFLVACQFLRVITFYSTQ	134
IPCS2	ETVFTSV-----FL-SFFLWTFHPFILKTKKIYTVLIWCRVLAFLVACQFLRVITFYSTQ	134
IPCS3	ETVFVTI-----FG-SFILWTFHPFVSHSKKICTVLIWCRVFVYLAASQSLRIITFFATQ	134
	* : . : * * : : * . : . : * : : . * : : . : ** *** : *	
PLM	LPSARPWCASARFNNVPSQPHRWAQKYVPYANDPAAIRKLLHWDAAAYADPGSYIGDYRA	237
IPCS1	LPGPNYHCREGSE LARLP RPHN-----	156
IPCS2	LPGPNYHCREGSKVSRLPWPKS-----	156
IPCS3	LPGPNYHCREGSKLAKIPPPKN-----	156
	** . . * . . * :	
PLM	DWGSMSFLEFLRPSYSEGSWFALLKKAGGGCNDLMYSGHMLVAVLTAMAWTE-AYGGF	296
IPCS1	-----VLEVLL-----LNFPRGVIYGCGLIFSSHMIFTLVFVRTYQYKYSKRF	200
IPCS2	-----ALEVLE-----IN-PHGVMYGCGLIFSSHMIFTLVFVRTYQYGTGRF	199
IPCS3	-----VLEVLL-----INFPDGVIYGCGLIFSSHTIFTLVFVRTYQRYGTTRW	200
	* , * : . ** , ** : , * . : : : . : : . : :	
PLM	SSAMIWLFVAHSAQR EIRERHHYTVDCIVAIVVGILLWKM-TGFIW-SAERKTKQTKLEK	354
IPCS1	IKLLGWVIAILQSLLIIASRKHYTVVVVAWYTVNLVVFCLDKKLELPDRTTAL-----	255
IPCS2	IKLFGWLTAIVQSLLIIASRKHYSDVVVAWYTVNLVVFCLDKKLELPDRTAVL-----	254
IPCS3	IKHLAWLMAVIQSILIIASRKHYTDIVVAWYTVNLVMFYVDSKLPEMAERSSGPS-PTP	259
	. : * : . . : * * : * : * : * : * : * : : * : :	
PLM	IQNSLIHAAGDGIETVRRLVEEIEVSSR-----VEKQSKVISNRTMTVFACATVITTLT	409
IPCS1	-LPVI---SKDRTKEESHKLLNGNGVDPADRRPRAQVNGK-DSNGGHTDNATNGT-----	305
IPCS2	-LPVI---SKDRTKEENHKLLNGNGVDPADWRPRAQVNGKIDSGNVHTDNTMNGA-----	305
IPCS3	LLPLSTKDSKNKSKEDHQRLLENNVADDH-----	289
	* : * : : * : : *	
PLM	IVILALTLTSDG	421
IPCS1	-----	305
IPCS2	-----	305
IPCS3	-----	289

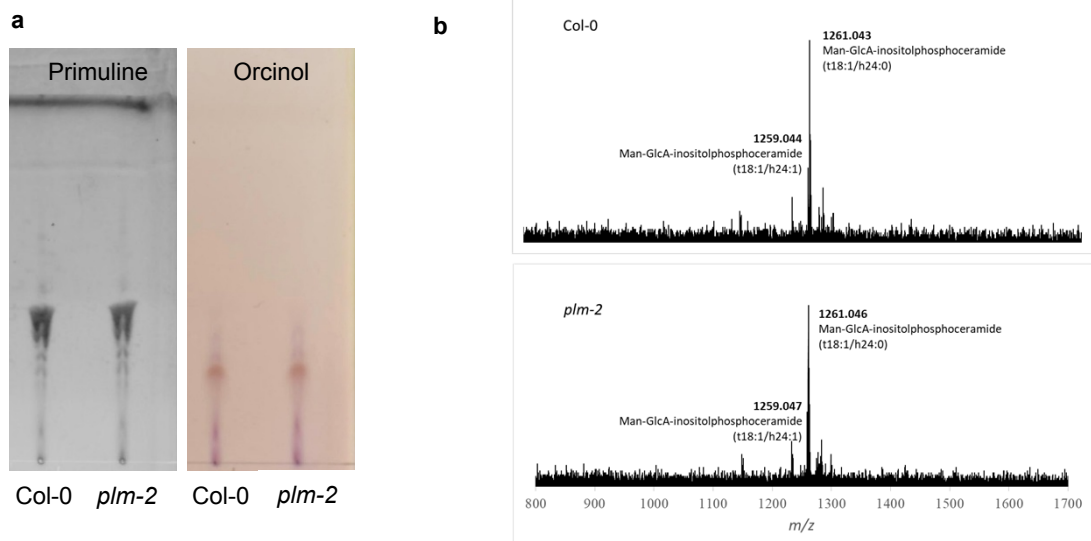
Supplementary Fig. 4. Protein Sequence alignment of PLM (At1g43580) and AtIPCSs. * identical residues. · similar residues.



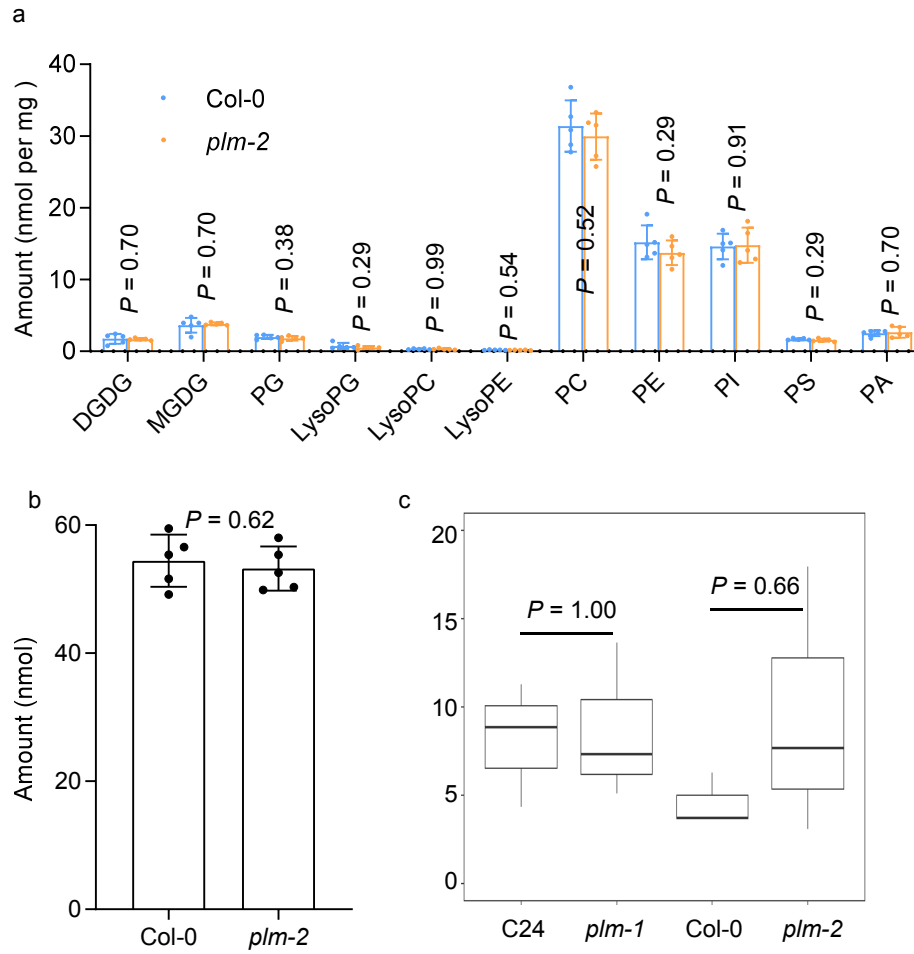
Supplementary Fig 5. Subcellular localization of PLM-YFP. **a.** Co-localization of PLM-YFP with RFP (RFP-HDEL) in leaf epidermal cells. **b.** Co-localization of PLM-YFP with RFP (RFP-HDEL) in hypocotyl epidermal cells. **c.** Transient co-expression of PLM-YFP with RTNLB6-RFP in the leaf cells of *Nicotiana benthamiana*. All the experiments were repeated independently three times with similar results.



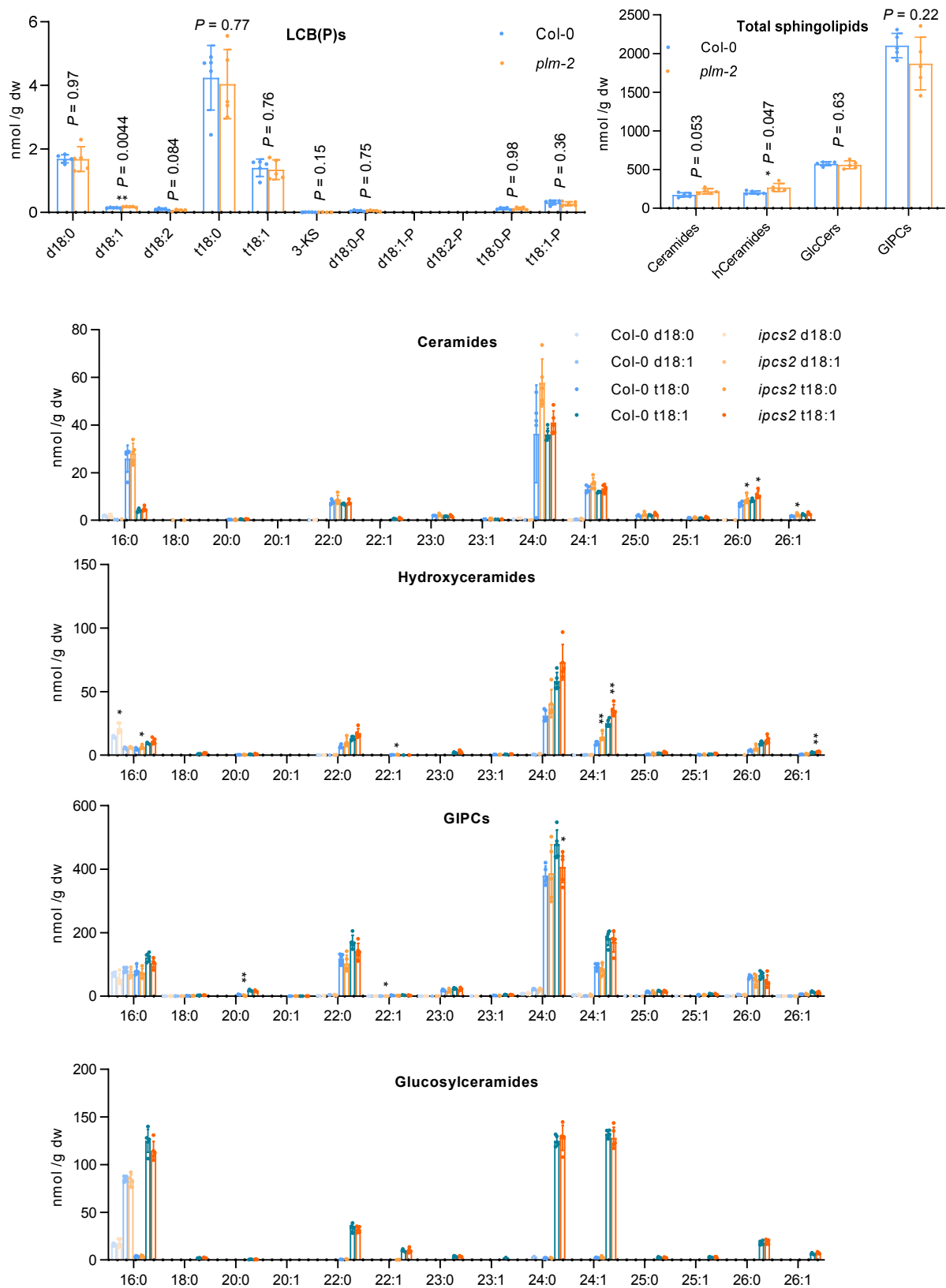
Supplementary Fig. 6. Repetition of LCB(P)s and complex sphingolipid species analysis in wild-type Col-0 and *plm-2*. Measurements are the average of five replicates (n = 5 independent samples). Data are means \pm SD. Statistically significant differences are indicated by two-tailed Student's t-test. *, $P < 0.05$. **, $P < 0.01$. hCeramides, hydroxyceramides. GlcCers, glucosylceramides.



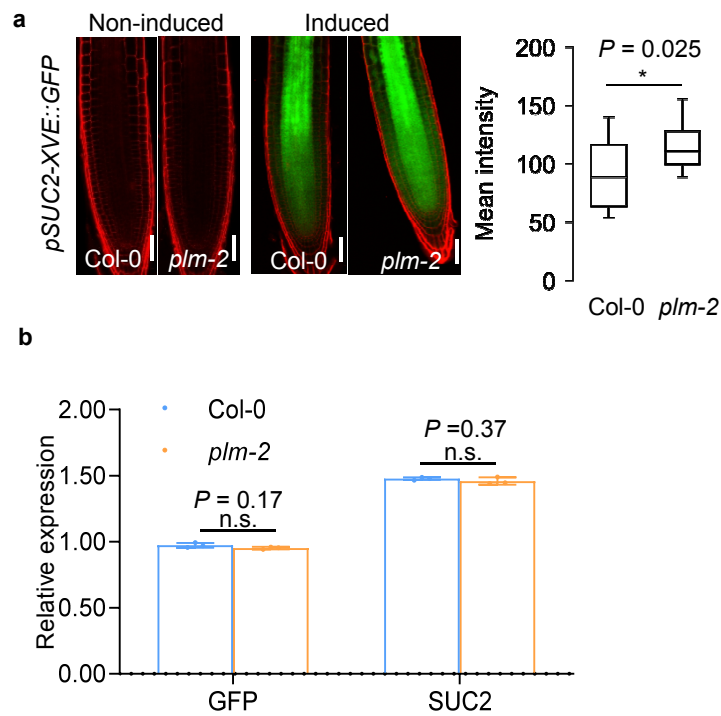
Supplementary Fig. 7. GIPC glycosylation assay. **a.** Thin layer chromatography (TLC) of an enriched GIPC fraction from 5-day-old roots stained with primuline (binding to certain lipid classes) and orcinol (revealing sugars). **b.** Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis of GIPC enriched fraction from 5-day-old roots to identify highly glycosylated GIPCs. All the experiments were repeated independently two times with similar results.



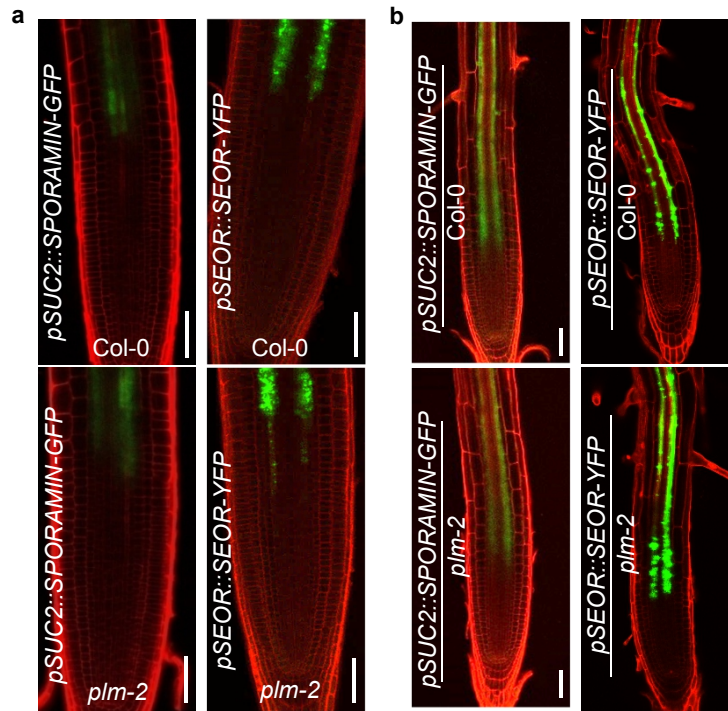
Supplementary Fig. 8. Plant routine lipids profile. **a.** Plant polar lipids assay in wild-type Col-0 and *plm-2*. Data are means \pm SD and based on five replicates ($n = 5$ independent samples). **b.** Relative amount of DAG in wild-type Col-0 and *plm-2*. Data are means \pm SD and based on five replicates with independent samples. **c.** The total sterol amount in 5-day-old seedlings of wild-type and *plm* mutants. Data are based on three replicates with independent samples. The boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values. The black lines within the boxes indicate the median values. Significant differences were determined by two-tailed Mann-Whitney-Wilcoxon test. No significances were found between wild type and *plm* mutants in the above assays.



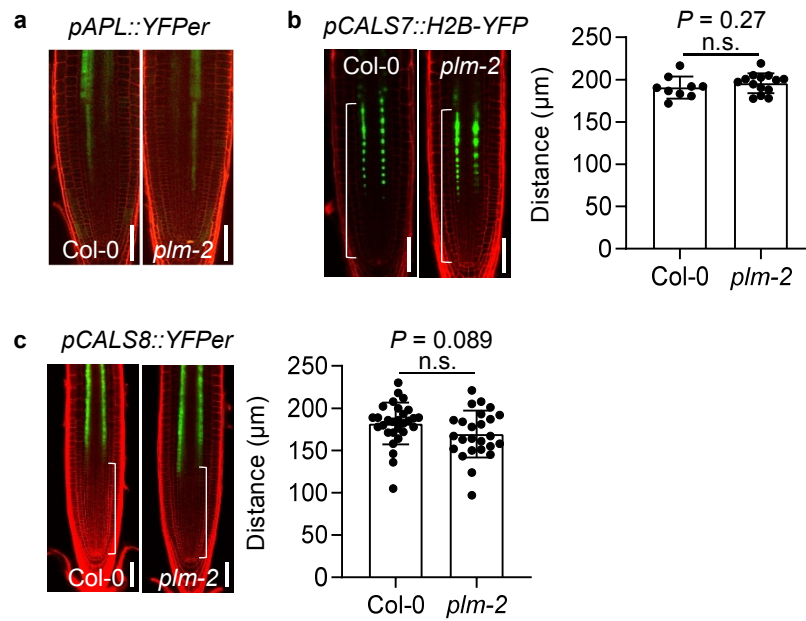
Supplementary Fig.9 Analysis of LCB(P)s and complex sphingolipid species in wild-type Col-0 and *ipcs2* (SALK_206784). Measurements are the average of five replicates ($n = 5$ independent samples). Data are means \pm SD. Statistically significant differences are indicated by two-tailed Student's *t*-test. *, $P < 0.05$. **, $P < 0.01$. hCeramides, hydroxyceramides. GlcCers, glucosylceramides.



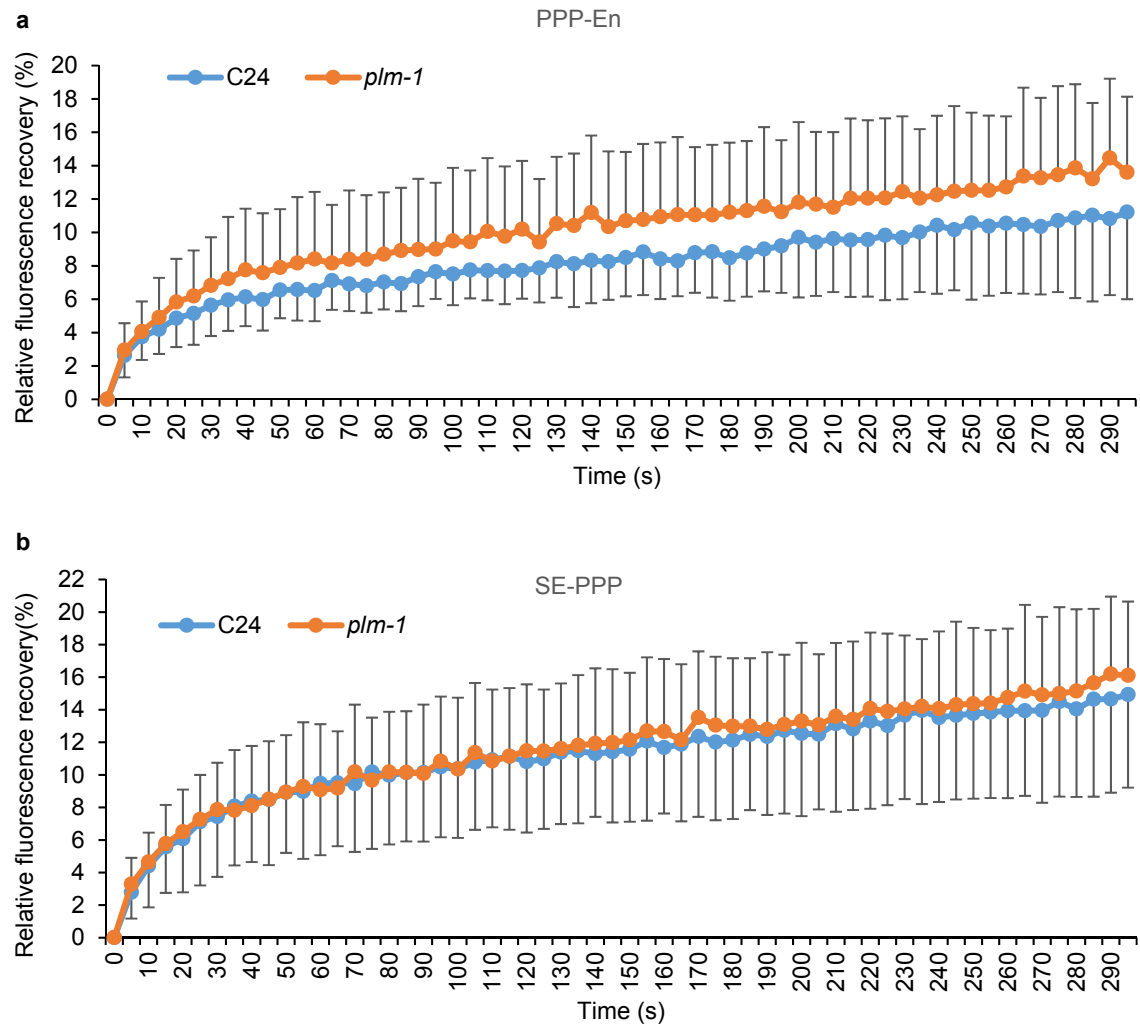
Supplementary Fig.10. *PLM* promotes GFP unloading without affecting *GFP* and *SUC2* expression. **a.** Col-0 and *plm-2* carrying inducible *pSUC2-XVE::GFP* upon 25h induction by 5 μM β -estradiol. An area of 50 x 40 μm^2 , 50 μm from QC, was used for fluorescence intensity quantification. $n = 15$ (Col-0), $n = 13$ (*plm-2*) biologically independent plants. The boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values. The black lines within the boxes indicate the median values. **b.** qRT-PCR for *GFP* and *SUC2* transcript levels in wild-type Col-0 and *plm-2*. Data are based on three independent experiments. Data are means \pm SD. Significant differences were determined by two-tailed Mann-Whitney-Wilcoxon test (a) and two-sided student's *t*-test (b). * $P < 0.05$. n.s., no significance. Scale bars, 50 μm (a).



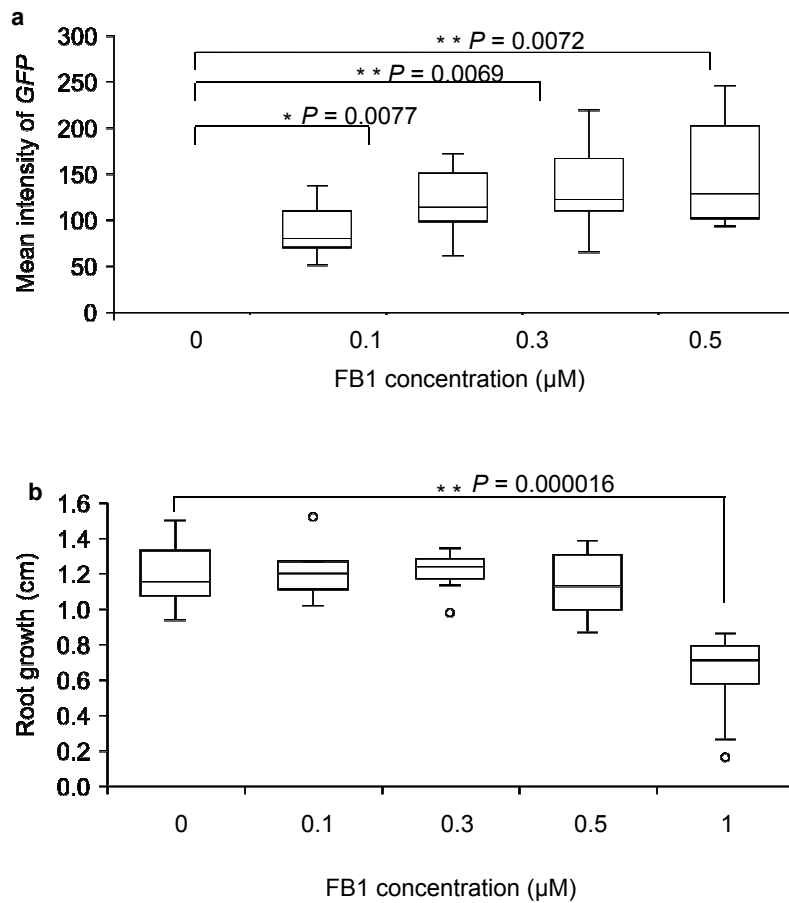
Supplementary Fig. 11. Unloading pattern of GFP fusion proteins with larger size in roots. a. *pSUC2::SPORAMIN-GFP* and *pSEOR::SEOR-YFP* in Col-0 and *plm-2*. **b.** Grafting assay using *pSUC2::SPORAMIN-GFP*²⁷ and *pSEOR::SEOR-YFP*²⁶ shoots as scions and Col-0 and *plm-2* roots as stocks. Scale bars, 50 μm (a,b). the experiment was repeated three times independently with similar results.



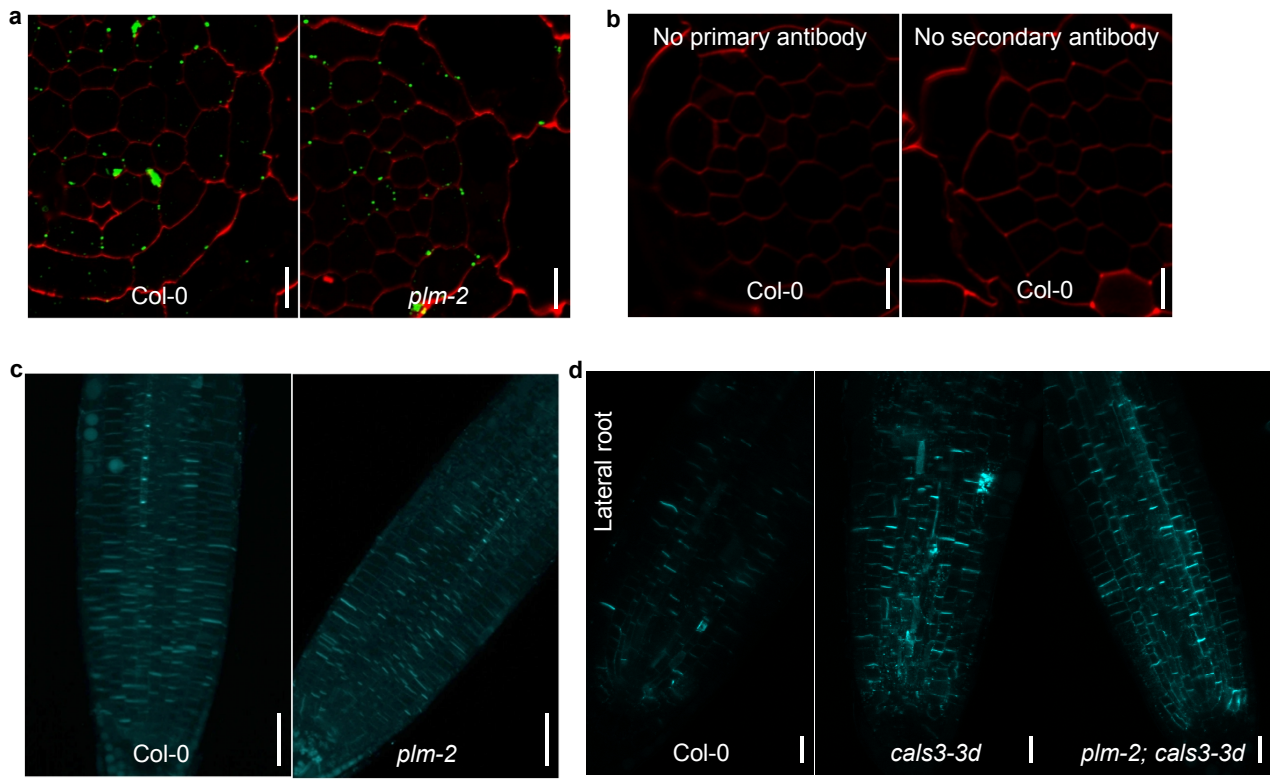
Supplementary Fig. 12. Root phloem development is not impaired by *plm* mutation. **a.** *pAPL::YFPer* in wild-type Col-0 and *plm-2*. The experiment was repeated four times independently with similar results. **b.** *pCALS7::H2B-YFP⁵¹* and the distances from QC to the first enucleated cell in wild-type Col-0 ($n = 9$) and *plm-2* ($n = 14$) independent plants. **c.** *pCALS8::YFPer* and the distances from QC to the first expressed cell in wild-type Col-0 ($n = 29$) and *plm-2* ($n = 25$) independent plants. Data represent means \pm SD. Significant differences were determined by two-tailed student's *t* test. Scale bars, 50 μm (**a,b,c**).



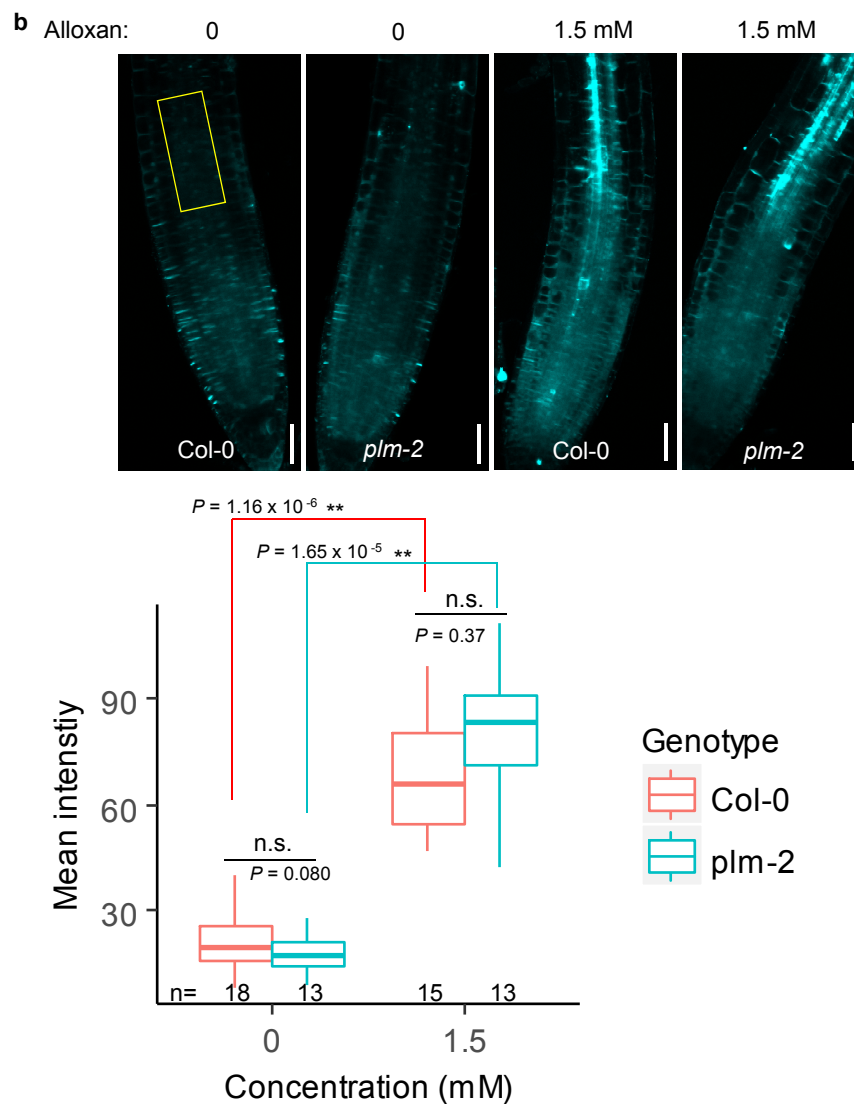
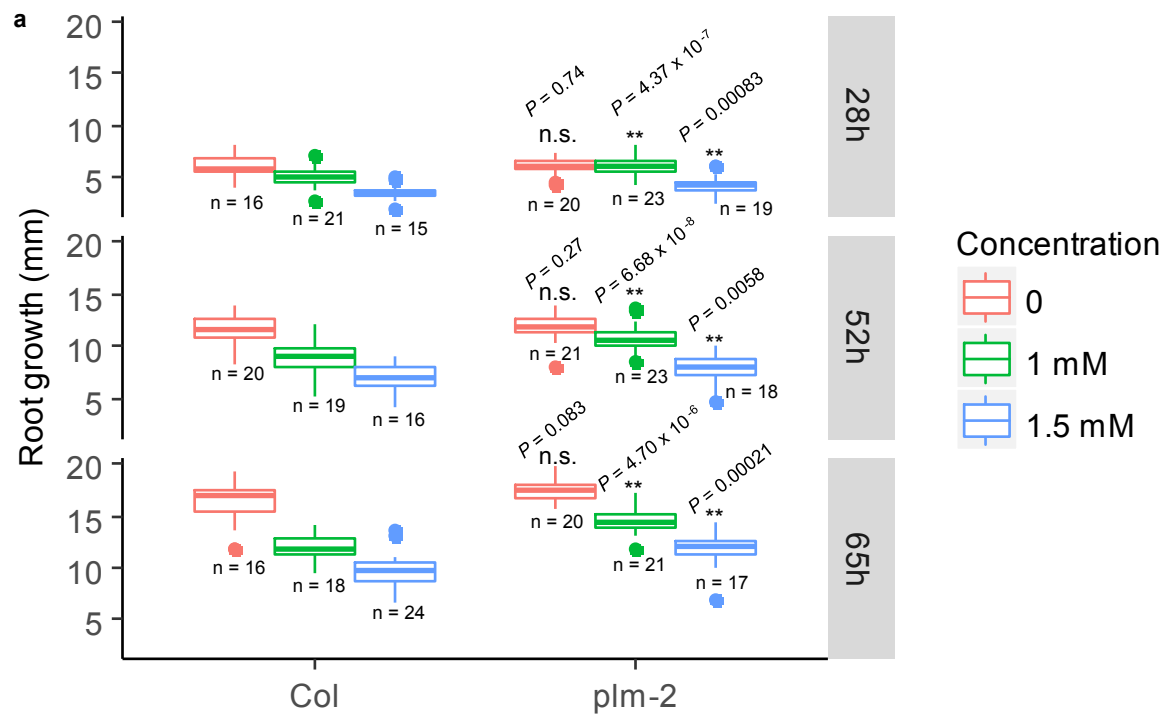
Supplementary Fig 13. FRAP assays in C24 and *plm-1* expressing *pSUC2::GFP*. **a.** GFP movement from PPP to endodermis post bleaching of outward layers in the unloading domain. n= 9 (C24) and 7 (*plm-1*) biologically independent samples. **b.** GFP movement from SE to PPP post bleaching of outward layers in the unloading domain. n= 7 (C24) and 7 (*plm-1*) independent plants. Data represent means \pm SD.



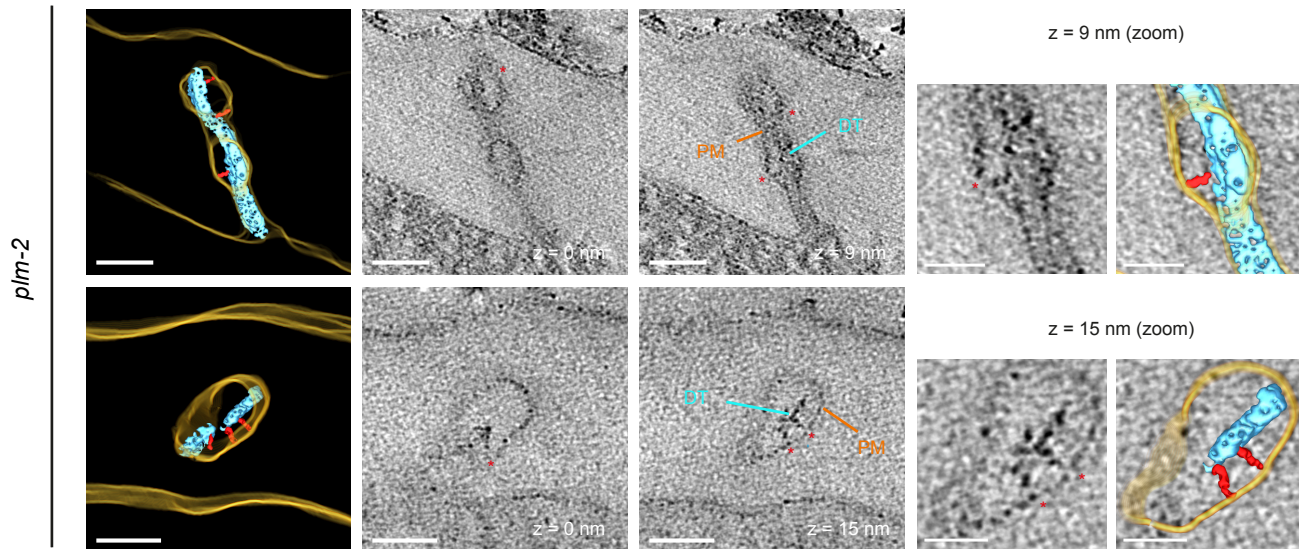
Supplementary Fig. 14. Roots with FB1 treatment phenocopies *p/m* in enhanced GFP unloading. **a.** Fluorescence intensity in the root meristem of wild-type Col-0 upon 72h treatment by different concentration of FB1. 3-day-old seedlings growing on normal media were used for treatment. An area of 50 x 40 μm², 50 μm from QC, was transferred for quantification. n = 16 (0 μM), n = 14 (0.1 μM), n = 14 (0.3 μM), n = 16 (0.5 μM) independent plants. **b.** Root growth of wild-type Col-0 upon 72h treatment by different concentration of FB1. 3-day-old seedlings growing on normal media were transferred for treatment. n = 10 independent plants for each concentration. The boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values. The black lines within the boxes indicate the median values. Outliers are shown as dots. Significant differences were determined by two-tailed Mann-Whitney-Wilcoxon test. *, $P < 0.05$. **, $P < 0.01$.



Supplementary Fig. 15. Callose deposition analysis. **a.** Callose deposition (green) in different cell layers shown by immunolocalization using callose antibody. **b.** Immunolocalization controls without primary and secondary antibody. **c.** Aniline blue stained primary roots of wild-type Col-0 and *plm-2*. **d.** Aniline blue stained lateral roots of wild-type Col-0, *cals3-3d* and *cals3-3d;plm-2*. All the experiments were repeated independently three times with similar results. Scale bars, 5 μm (a,b), 50 μm (c,d).



Supplementary Fig. 16. Root growth assay by alloxan treatment. 5-day-old seedlings were transferred to same media added with 0, 1, 1.5mM alloxan and the root elongation at 28, 52 and 65h was measured. **b.** Callose accumulation in roots by aniline blue staining at 28h after transferred to media with 0 and 1.5 mM alloxan. The experiment is repeated three times independently with similar results. A $138 \times 60 \mu\text{m}$ area in the central part starting from transition zone was used for intensity quantification. The boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values. The black lines within the boxes indicate the median values. Outliers are shown as dots. Independent plant numbers (n) are indicated in the graphs. Significant differences between Col-0 and *plm-2* under same conditions were determined by two-tailed Mann-Whitney-Wilcoxon test. ** $P < 0.01$. n.s., no significance. Scale bars, $50 \mu\text{m}$ (b).



Supplementary Fig. 17. *plm-2* still possesses type II plasmodesmata at the SE-PPP interface of the unloading zone.

Examples of type II plasmodesmata in *plm-2* roots detected with electron tomography at the SE-PPP interface, within the unloading domain. Due to the large size of these PDs the full volume was not contained within the section. Reconstructions are therefore partial with missing volumes. Plasmodesmata are not classified by morphology due to missing volumes that would make the calls arbitrary. In general, they were branched in nature. Panels with black background are 3D reconstructions based on 2D image stacks. Plasma membrane (PM) is rendered in yellow, desmotubule (DT) and ER in light blue, tethers within plasmodesmata in red. Selected 2D views are displayed in the neighbouring panels with zooms for tethers within type II plasmodesmata (with a model overlay). Distance to the first 2D view is indicated under or above each panel. Arrows, symbols and labels indicate various plasmodesmata components and follow the colour scheme of the 3D models (* tether elements within plasmodesmata). Scale bars are of 50nm or 25nm for the zoomed images. Experiment was repeated two times independently with similar results.

Supplementary Table 1. Primer used in this study.

Primer name	Sequence	Used for
MN1.5-F	TTATTATCAAGATCAAAGATTGTATGGTTT	Linked markers for mapping
MN1.5-R	CTTGTTTTTATATCTGTTTGTTTAATTGT	
CIW12-F	AGGTTTTATTGCTTTTCACA	
CIW12-R	CTTTCAAAAGCACATCACA	
NGA280-F	GGCTCCATAAAAAAGTGCACC	
NGA280-R	CTGATCTCACGGACAATAGTGC	
cals3-1d-inner-F	CATAGATTGGATCCTACCTCCAGTGGCAA	Mutation genotyping
cals3-1d-inner-R	AGTGCAGTCTTAAACTGCCGAACACATC	
cals3-1d-outer-F	GCAGCGGCGGATTATACGAACTCA G	
cals3-1d-outer-R	CCCTGCCCATCAGTGTTGGATCATGTT	
cals3-3d-inner-F	GTTGCAGAAAAGACACAGTTGTATGTACT	Mutation genotyping
cals3-3d-inner-R	AGGATCGAGAGGTAGGATATTATCGG	
cals3-3d-outer-F	ATGCTGCTGATAAGGCTGACC	
cals3-3d-outer-R	AGAGCAAGAACAGCAGCTTGG	
plm-2-F	TCTGAAGGGTCTTCTTGTTTGC	Genotyping
plm-2-R	TTTAGCCGTCGCTTGTCAAAGT	
LBb1.3	ATTTTGCCGATTTCCGAAC	
PLM-m-F	CGGGACACTTGTGGAAATCC	RT-PCR
PLM-m-R	AGTCAATGCCAGAATCACTA	
PLM-p-attB4	GGGGACAACTTTGTATAGAAAAGTTGTGAGTTTTACTTTATAC TTCTAAC	<i>PLM</i> promoter
PLM-p-attB1r	GGGGACTGCTTTTTTGTACAAACTTGGAAGTGGGTTTAATTA AGCTAA	
PLM-g-attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGACTAAAG GTGGACTTGG	<i>PLM</i> genomic sequence
PLM-g-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGCCGTCGCTTG TCAAAGTCA	
iSUC2-F	GGGGTACCCCTTGCGGTGGTTGTAGTGGTGGAG	Inducible <i>SUC2</i> promoter
iSUC2-R	CCCTCGAGCCACTTTGTTTTGTGGGAGAC	
SUC2-m-F	GCAGACGGGTGAGTTAGA	qPCR
SUC2-m-R	GGAGATTGGACCACAGAG	
GFP-m-F	AAGCTGACCCTGAAGTTCATCTGC	qPCR
GFP-m-R	CTTGTAGTTGCCGTCGTCCTTGAA	

F, forward. R, reversed.

Supplementary Table 2. Protein sequences for phylogenetic tree in Figure.2b.

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>Q8NHU3 SMS2_Homo sapiens

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>Q96LT4 SMSr_Homo sapiens

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>E7Q687 Aur1p_Saccharomyces cerevisiae

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>Q9VS60O SMSr_Drosophila melanogaster

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>Q20735 SMS2_Caenorhabditis elegans

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>Q9SH93 IPCS2_Arabidopsis thaliana

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>Q56Y01 IPCS3_Arabidopsis thaliana

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Supplementary Table 3. P values (t-test) of sphingolipid profile analysis. n.d.,no data.

Fig. 3c. C24 and *plm-1*.

Ceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	6.85E-01	n.d.	n.d.	n.d.	3.95E-02	n.d.	n.d.	n.d.	1.39E-01	4.00E-03	1.48E-01	n.d.	3.60E-02	n.d.
d18:1	8.57E-01	7.06E-01	n.d.	n.d.	n.d.	7.22E-01	n.d.	n.d.	1.30E-02	4.60E-01	n.d.	n.d.	n.d.	n.d.
t18:0	9.81E-01	7.67E-01	3.71E-01	n.d.	3.00E-02	8.74E-01	5.71E-02	8.07E-01	2.04E-03	5.34E-04	3.90E-02	1.34E-02	1.26E-03	2.42E-04
t18:1	3.19E-01	5.84E-01	1.07E-01	n.d.	8.76E-04	4.45E-01	1.27E-02	8.24E-01	7.10E-05	2.23E-05	3.06E-03	6.84E-03	1.58E-03	2.74E-04
Hydroxyceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	1.55E-01	n.d.	n.d.	n.d.	6.68E-01	n.d.	n.d.	n.d.	9.83E-01	3.23E-01	n.d.	n.d.	1.56E-01	n.d.
d18:1	3.49E-03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.74E-01	4.53E-01	n.d.	n.d.	n.d.	n.d.
t18:0	8.51E-01	9.27E-01	4.20E-01	n.d.	8.02E-01	1.51E-01	3.94E-01	5.66E-01	6.60E-01	7.63E-01	9.17E-01	2.46E-01	8.79E-01	5.29E-01
t18:1	2.01E-01	2.54E-01	6.89E-01	n.d.	5.79E-02	2.61E-03	6.24E-02	1.95E-02	5.01E-02	1.36E-01	1.62E-02	1.43E-01	5.87E-02	2.49E-01
GIPCs	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	8.75E-01	2.11E-01	1.62E-01	n.d.	6.45E-01	n.d.	2.45E-01	n.d.	9.35E-01	9.73E-01	n.d.	n.d.	5.83E-01	7.48E-01
d18:1	2.35E-02	6.49E-01	9.04E-01	n.d.	4.45E-02	n.d.	n.d.	n.d.	1.91E-02	8.27E-04	4.65E-01	9.64E-01	2.58E-01	2.44E-01
t18:0	6.54E-01	8.69E-02	1.62E-01	1.03E-01	9.24E-01	9.77E-01	4.47E-01	3.25E-01	7.74E-01	5.25E-01	7.46E-01	3.92E-01	6.87E-01	9.61E-01
t18:1	2.27E-02	3.22E-01	1.31E-01	1.40E-02	1.22E-02	1.21E-02	5.82E-02	4.37E-02	2.67E-02	9.07E-02	1.95E-01	4.53E-02	2.94E-01	2.52E-01
Glucosylceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	6.65E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	3.11E-03	3.72E-01	6.55E-01	n.d.	6.09E-02	n.d.	n.d.	n.d.	3.38E-02	9.85E-02	n.d.	n.d.	2.17E-01	7.43E-02
t18:0	7.24E-01	n.d.	n.d.	n.d.	5.21E-01	n.d.	5.63E-01	n.d.	6.45E-01	2.50E-01	n.d.	n.d.	7.45E-01	8.14E-01
t18:1	1.34E-02	5.85E-01	6.77E-02	n.d.	2.32E-02	3.69E-03	5.07E-02	1.35E-02	1.04E-02	1.88E-03	1.66E-01	2.10E-02	1.08E-01	3.63E-01

Fig. 3d. Col-0 and *plm-2*.

Ceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	2.51E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.21E-01	n.d.	n.d.	n.d.	n.d.
t18:0	5.37E-01	n.d.	1.15E-01	n.d.	6.58E-02	n.d.	2.67E-03	1.92E-01	1.46E-04	2.90E-04	8.37E-03	9.70E-03	1.70E-04	2.89E-04
t18:1	4.70E-01	n.d.	7.22E-01	n.d.	7.71E-03	3.78E-02	1.30E-04	2.49E-02	4.09E-06	9.32E-07	3.80E-04	6.07E-03	3.50E-06	5.44E-05
Hydroxyceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	7.16E-01	n.d.	n.d.	n.d.	7.74E-03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	5.06E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.58E-01	6.32E-01	n.d.	n.d.	n.d.	n.d.
t18:0	1.96E-01	n.d.	1.87E-01	n.d.	4.28E-02	5.20E-02	n.d.	6.50E-01	9.37E-02	5.72E-02	9.04E-02	3.59E-01	1.36E-01	8.40E-01
t18:1	1.48E-02	4.16E-03	1.18E-02	n.d.	2.08E-02	n.d.	5.38E-02	1.73E-02	3.51E-02	1.28E-02	6.29E-02	2.89E-02	4.51E-02	1.02E-01
GIPCs	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	9.69E-02	2.65E-01	n.d.	n.d.	1.01E-02	n.d.	6.78E-01	8.72E-01	3.32E-03	4.89E-03	n.d.	n.d.	5.82E-01	4.73E-02
d18:1	3.07E-01	5.53E-01	n.d.	n.d.	5.70E-02	1.30E-01	n.d.	n.d.	3.27E-03	9.70E-01	n.d.	n.d.	2.73E-04	7.80E-04
t18:0	7.12E-02	2.38E-02	1.86E-02	9.72E-02	1.97E-02	1.77E-02	9.30E-03	3.87E-02	2.31E-03	1.92E-03	5.05E-03	2.86E-02	3.18E-05	2.00E-04
t18:1	2.82E-02	3.53E-02	8.86E-02	2.18E-01	3.08E-02	5.16E-01	1.08E-02	4.99E-02	3.81E-03	8.59E-03	7.30E-04	1.66E-03	3.21E-02	2.29E-03
Glucosylceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	6.39E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	2.83E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
t18:0	1.32E-01	n.d.	n.d.	n.d.	5.78E-03	n.d.	n.d.	n.d.	3.48E-01	4.98E-01	n.d.	n.d.	n.d.	n.d.
t18:1	7.62E-01	4.63E-02	2.41E-02	n.d.	2.80E-01	7.20E-04	6.96E-02	n.d.	2.20E-02	2.42E-01	5.02E-01	1.37E-01	6.03E-02	8.71E-01

Fig. S6. Col-0 and *plm-2*.

Ceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	5.91E-01	n.d.	n.d.	n.d.	1.94E-01	n.d.	7.58E-01	n.d.	5.16E-01	5.78E-01	5.53E-01	n.d.	5.17E-01	n.d.
d18:1	1.17E-01	5.27E-01	n.d.	n.d.	n.d.	6.33E-01	n.d.	n.d.	1.09E-01	3.69E-02	n.d.	n.d.	1.36E-01	n.d.
t18:0	1.67E-01	2.15E-01	6.72E-01	n.d.	2.17E-02	7.71E-01	3.15E-03	1.22E-01	1.95E-04	3.64E-04	5.97E-04	1.30E-04	9.20E-04	1.18E-04
t18:1	2.66E-01	1.23E-01	2.47E-01	6.99E-01	6.61E-02	3.23E-01	1.33E-03	9.20E-01	3.59E-03	1.60E-03	4.24E-04	8.44E-04	4.71E-04	2.55E-04
Hydroxyceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	6.28E-03	n.d.	n.d.	n.d.	7.41E-01	n.d.	n.d.	n.d.	9.48E-01	4.55E-02	n.d.	n.d.	7.33E-01	n.d.
d18:1	6.15E-02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.22E-01	3.87E-01	n.d.	n.d.	n.d.	n.d.
t18:0	5.43E-01	7.73E-02	1.96E-01	n.d.	6.39E-01	4.61E-01	5.56E-01	5.94E-01	8.85E-01	5.04E-02	4.35E-02	9.40E-02	3.47E-01	3.82E-02
t18:1	9.08E-01	5.81E-01	8.41E-01	6.90E-01	9.33E-01	7.39E-01	6.17E-01	6.82E-01	7.91E-01	5.20E-02	7.21E-01	3.17E-01	3.89E-01	9.67E-02
GIPCs	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	5.25E-01	1.15E-01	3.81E-01	n.d.	4.34E-01	n.d.	3.90E-01	n.d.	2.43E-01	3.19E-02	5.51E-01	n.d.	3.04E-01	4.23E-02
d18:1	5.73E-01	4.09E-01	5.00E-01	n.d.	9.25E-01	n.d.	n.d.	n.d.	6.21E-02	5.14E-03	n.d.	2.70E-01	2.52E-01	1.37E-01
t18:0	3.69E-01	6.62E-01	6.25E-01	4.71E-03	8.98E-01	7.09E-01	8.14E-01	9.42E-01	4.53E-01	3.62E-01	7.21E-01	8.13E-01	4.39E-01	7.12E-01
t18:1	8.46E-01	8.75E-02	2.05E-01	5.41E-03	8.21E-01	1.32E-01	5.58E-01	1.25E-01	3.30E-01	1.66E-01	3.11E-01	2.38E-01	3.36E-01	2.13E-01
Glucosylceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1

d18:0	1.07E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	3.50E-01	6.41E-01	9.60E-01	n.d.	1.38E-01	n.d.	n.d.	n.d.	3.10E-01	4.54E-01	n.d.	n.d.	2.68E-01	7.09E-02
t18:0	7.04E-02	n.d.	n.d.	n.d.	9.46E-02	8.38E-02	n.d.	n.d.	2.28E-01	1.84E-01	n.d.	n.d.	2.05E-01	9.67E-01
t18:1	2.36E-01	2.24E-01	7.28E-02	n.d.	1.63E-01	1.03E-01	1.69E-01	1.46E-01	2.80E-01	4.53E-01	2.99E-01	2.23E-01	1.01E-01	5.01E-01

Fig. S9. Col-0 and *ipcs2*.

Ceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	6.79E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.72E-02	n.d.	n.d.	n.d.	n.d.
t18:0	5.04E-01	n.d.	7.60E-01	n.d.	3.23E-01	n.d.	2.98E-01	4.57E-01	8.15E-02	8.61E-02	4.10E-01	9.42E-01	4.94E-02	4.90E-02
t18:1	2.72E-01	n.d.	2.08E-01	n.d.	3.08E-01	2.33E-01	9.32E-01	9.28E-01	9.43E-02	9.94E-02	1.41E-01	6.44E-02	2.21E-02	9.31E-02
Hydroxyceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	1.35E-02	n.d.	n.d.	n.d.	5.32E-02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	2.03E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.45E-01	7.86E-01	n.d.	n.d.	n.d.	n.d.
t18:0	4.97E-02	n.d.	1.52E-01	n.d.	9.47E-02	2.09E-02	n.d.	6.02E-01	1.28E-01	9.81E-03	2.07E-01	3.64E-01	1.29E-01	6.22E-02
t18:1	1.26E-01	6.88E-02	1.40E-01	n.d.	6.28E-02	n.d.	1.53E-01	6.51E-02	7.90E-02	3.67E-03	6.06E-02	5.36E-02	1.21E-01	3.42E-03
GIPCs	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	1.10E-01	9.71E-02	n.d.	n.d.	2.99E-01	2.33E-01	9.33E-01	1.76E-01	9.88E-01	5.75E-01	n.d.	n.d.	1.69E-01	2.84E-01
d18:1	1.18E-01	7.55E-01	n.d.	n.d.	3.86E-01	1.68E-02	n.d.	n.d.	7.34E-01	6.83E-02	n.d.	n.d.	1.17E-01	2.93E-01
t18:0	3.64E-01	3.89E-01	2.39E-03	4.07E-01	2.91E-01	1.91E-01	4.09E-01	3.39E-01	8.72E-01	1.98E-01	6.37E-01	4.92E-01	2.60E-01	9.52E-01
t18:1	7.66E-02	1.56E-01	1.49E-01	5.82E-01	8.49E-02	1.30E-01	5.64E-01	1.48E-01	4.18E-02	5.26E-01	1.86E-01	1.79E-01	9.61E-02	5.28E-01
Glucosylceramides	16:0	18:0	20:0	20:1	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26:0	26:1
d18:0	4.48E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1	4.79E-01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
t18:0	9.65E-01	n.d.	n.d.	n.d.	8.31E-01	n.d.	n.d.	n.d.	8.05E-01	6.09E-01	n.d.	n.d.	n.d.	n.d.
t18:1	1.70E-01	2.65E-01	2.12E-01	n.d.	4.05E-01	9.00E-01	7.17E-01	n.d.	6.30E-01	4.47E-01	8.33E-01	4.97E-01	4.98E-01	1.66E-01